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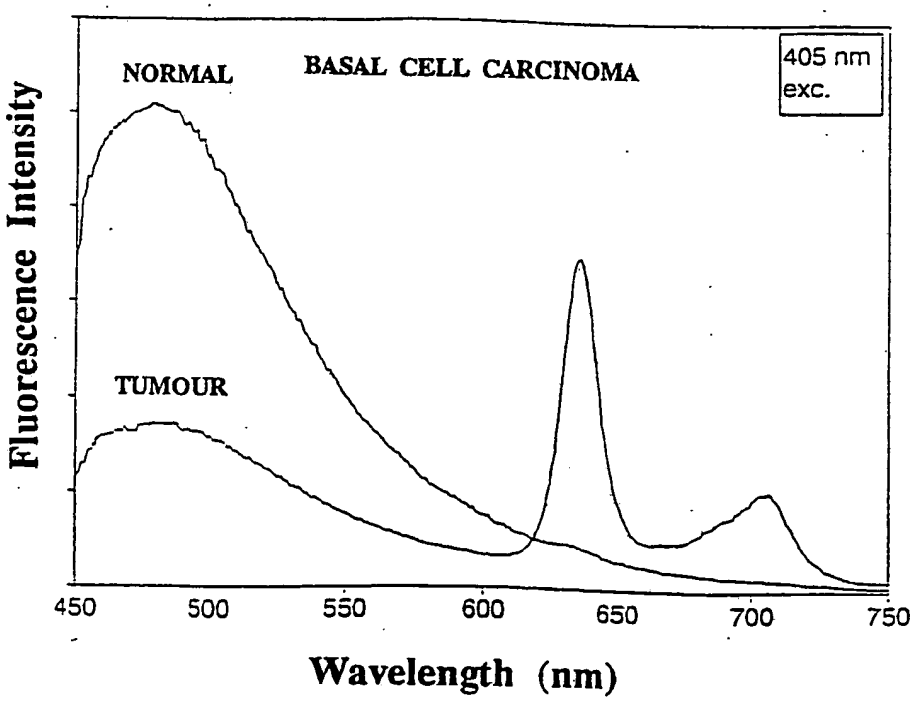
(54) Title: FLUORESCENCE DIAGNOSTICS OF CANCER USING δ-AMINO LEVULINIC ACID

(57) Abstract

A procedure for the detection of malignant tumours using the agent δ-amino levulinic acid (hydrochloride) as a tumour marker is described. The tissue is exposed to the agent either by intravenous injection or by application of an unguent over superficial tumours. After a suitable time delay fluorescence is induced in the tissue using an excitation source, e.g. a laser. Cancer then emerges with a strong and specific fluorescence peak at 635 nm. By dividing this red peak intensity by the intensity of the blue-green autofluorescence from the tissue an improved demarcation is obtained and also immunity to variations in many experimental parameters.



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Fluorescence diagnostics of cancer using δ-amino levulinic acid

Field of the invention

Cancer diseases are very widespread and have a major social and socio-economic impact. The incidence of cancer has shown a steady increase during the last tens of years. It is generally accepted that an early cancer diagnosis and a radical treatment is of great importance for the prognosis for the patient. Conventional diagnostic methods for cancer comprise different types of X-ray investigations, endoscopic investigations with biopsy sampling in the hollow organs of the body, cytological puncture of resistances, as well as smear cytology. The present invention describes the use of δ-amino levulinic acid (abbreviated ALA below) as a specifically tumour seeking agent, to be visualized through its specific fluorescence on irradiation with a laser or other suitable radiation source. A point monitoring or an imaging fluorescence system can be used for the detection. ALA, intravenously administered, or applied as an unguent over superficial tumours, is very selectively taken up by tumour cells, in which the substance is transformed into protoporphyrin IX, which exhibits a very strong and characteristic fluorescence in the red wavelength region. This procedure can be used, e.g. for the detection of early bronchial or bladder cancer and for the demarcation of the extension of a tumour for assuring a radical tumour treatment.

Background for the invention

Since long it is known that certain agent that are intravenously injected can be accumulated in tumours. If photo sensitizing agents are utilized such a tumour marking can be used in two ways, for diagnostics thorugh the fluorescence of the agent and for tumour treatment by photodynamic techniques, where the photo-excited agent transfers its energy to oxygen molecules, that make

a transition from their normal triplet state to an aggressive singlet state. The release of singlet oxygen selectively in the tumour causes tissue necrosis. During the last few years the commercially available agent Photofrin (Quadra Logic Technologies, Vancouver, Canada) has been injected and used clinically for the double purpose just described. Experimentally it has been found, that the selectivity for a tumour over surrounding tissue seldom exceeds a factor of 2-3, which limits the power of the method both for diagnostics and for therapy. It is possible to increase the discrimination for diagnostics by using also the autofluorescence of the tissue in the blue-green spectral region. This is utilized in procedures and equipment described in the Swedish patent # 455646 (Fluorescence device) and the European patent application # 0411104 (Improvements in diagnosis by means of fluorescent light emission from tissue). Also other agents, such as phtalocyanines, chlorines, purpurines and bensoporphyrin exhibit an accumulation in tumour tissue, but also for these the selectivity is limited, which reduces their value. Normal sensitizers have the difficulty that also the skin generally is sensitized, calling for the patient to be protected from strong day light for a period of about 4 weeks.

Description of the invention

The present invention is based on our observation, in animal experiments as well as clinically, that the agent δ-amino levulinic acid is an extremely efficient fluorescence marker for tumours. This is true for intravenous injection as well as for superficial treatment with ALA unguent direct on the tumour. The small non-fluorescing ALA molecules penetrate the cell membrane of malignant tumour cells and undergo in the mitochondria parts of the haem cycle, leading to the formation of protoporphyrin IX. Fluorescence can be induced by illumination by radiation from, e.g. a pulsed laser. This can be a dye laser pumped by a nitrogen laser. An especially suitable excitation wavelength is 405 nm, where porphyrins have their absorption maximum. The radiation can be conducted to the tissue through a quartz fibre, which can also be used to collect fluorescence light and to conduct it to a

spectral analyzer. This can be a spectrometer equipped with a diode-array detector or a more simple instrument based on optical filters. Tumours are marked by the occurrence of a fluorescence peak at 635 nm and a weaker peak at 710 nm. A demarcation ratio (the ratio between the specific fluorescence signal in a tumour and the signal in close-lying normal tissue) of about 10 or better can be obtained using this naturally occurring molecule in the body. It has no known side effects. A strong tumour demarcation is obtained after intra-venous injection if the investigation is performed about 1/2 hour after injection. ALA can also be prepared in an unguent base (about 20 % ALA) and be spread over superficial tumours and be kept in position using an occlusion bandage. The fluorescence investigation is performed 2-8 hours after the application.

Apart from exhibiting an increase in the specific red fluorescence, a decrease in the blue-green fluorescence from endogenous chromophores is observed in tumours. An especially effective form of diagnostics for ultraviolet or violet excitation is to evaluate the background-free fluorescence at 635 nm and divide it by the fluorescence intensity at about 470 nm. Such a ratio is dimensionless and therefore the signal will be independent of the measurement distance, the angle of incidence for the light, and fluctuations in the excitation and detection equipment.

The procedure given in this invention can also be used in combination with an imaging cancer detection system, which has been described in the Swedish patent # 455646. A simultaneous recording of images in three different fluorescence colours is then made (635 nm, 600 nm and 470 nm). An image intensifier, which is activated in synchronism with the exciting light source, is used for the recording. A new, generalized and tumour enhancing image is obtained by subtracting the 600 nm image (background) from the 635 nm image, followed by a division of the result by the blue image, pixel by pixel.

Cancer detection with fluorescence techiques using ALA is illustrated in Figure 1, where 1 indicates the excitation source, 2 indicates optical components to bring the exciting light to the tissue, 3 indicates the tissue that has been exposed to ALA, 4 indicates optical components for conducting the fluorescence light to a wavelength-dividing system 5 placed in front of the detection system 6. Finally, 7 indicates a signal processing system for tumour recognition based on the fluorescence properties.

The system proposed enables strongly improved possibilities for early detection of malignant tumours, also small tumours in the hollow organs of the body, and for clear demarcation of diseased tissue from surrounding normal tissue. No side effects occur.

The efficiency for this form of cancer detection is illustrated by the following three non-restricting examples.

Example 1.

A patient with basal cell carcinoma of the forehead was investigated with the method described in this invention. 6 hours before the fluorescence investigation the tumour areas were exposed to an unguent containing ALA in hydrochloric form (Porphyrin Products, Inc., Logan, Utah; catalogue # A 167). The unguent also covered a 10 mm wide zone of normal tissue. The fluorescence detection equipment used, as an excitation source, a Laser Science model VSL 337 pulsed nitrogen laser, pumping a Laser Science dye laser operating at a wavelength of 405 nm. The radiation from the dye laser was focussed by a lens into a quartz fibre. The other end of this fibre was put in contact with the tissue. Fluorescence light was collected through the same fibre, and after passing a dichroic beam splitter, used for injecting the laser light into the fibre, the fluorescence light was focussed on the entrance slit of a Jobin-Yvon 0.25 m grating monochromator equipped with an EG&G Optical Multichannel Analyser (OMA) III system. Fluorescence spectra from a basal cell cancer tumour and

from close-lying, ALA-exposed normal skin are shown in Figure 2. Using the intensity of the characteristic red fluorescence a demarcation ratio of 50 between the tumour and the normal tissue is obtained. If instead the ratio between the red and the blue-green fluorescence is used a demarcation ratio of 150 is obtained.

Example 2.

A patient with widespread local metastases of breast cancer was investigated with the same equipment and the same procedure as was described in Example 1. In Figure 3 fluorescence recordings 6 hours after application of the ALA unguent are shown for a skin area with lymphangitic metastatic breast cancer growth and for a close-lying normal skin area, also exposed to the ALA unguent. In this case the demarcation ratio is 13 and 25, respectively.

Example 3.

An experimental animal tumour model (human adenocarcinoma induced in the muscle of Wistar/Furth rats) was investigated with the method described in the present invention. The rat was intravenously injected with ALA hydrochloride in saline at a concentration of 30 mg/kg bodyweight. The rat was killed 30 minutes after the injection and the exposed tumour and the surrounding muscle were investigated using the same fluorescence set-up as was described in Example 1. Fluorescence signals from tumour and surrounding muscle are shown in Figure 4. A demarcation ratio of 9 is obtained using the characteristic red fluorescence. In this particular case, a division by the blue fluorescence intensity does not yield any further demarcation, for the excitation wavelength chosen, but still the advantages of recording a dimensionless quantity, as discussed above, are retained.

Figure captions

Figure 1. Schematic arrangment for fluorescence detection with ALA.

Figure 2. Fluorescence recordings for basal cell carcinoma and normal tissue for a patient that was exposed to an ALA unguent in the tumour area.

Figure 3. Fluorescence recordings for metastasis of breast cancer and normal tissue for a patient that was exposed to an ALA unguent in the tumour area.

Figure 4. Fluorescence recordings for an experimental animal tumour and surrounding normal tissue after intravenous injection of ALA.

Claims

1. A method for the detection of malignant tumours by fluorescence **characterized** by a substance, δ-aminolevulinic acid, which is supplied to the tissue and that the ratio between the specific peak of fluorescence intensity in red and in the blue-green is used to enhance the detection of tumours in investigations where the excitation is made in the ultra violet, violet or blue spectral range.

2. The method as recited in claim 1, **characterized** in that the applied substance is δ-aminolevulinic acid hydro-chloride.

3. The method as recited in claim 1, **characterized** in that the substance is a salve which is applied on to the suspicious malignant tissue.

4. The method as recited in claim 3, **characterized** in that the fluorescense examination is performed between 2 and 8 hours after the salve is applied.

5. The method as recited in claim 1, **characterized** in that the substance is intravenously administered.

6. The method as recited in claim 5, **characterized** in that the substance is injected with a concentration of 0.5 to 50 mg per kilo body weight.

7. The method as recited in claim 5, **characterized** in that the fluorescence examination is performed between 15 minutes to 2 hours after the injection.

8. The method as recited in claim 1, **characterized** in that the fluorescence examination is performed with laser excitation.

9. The method as recited in claim 1, **characterized** in that the fluorescence examination is performed with a conventional light source.

10. The method as recited in claim 1, **characterized** in that the fluorescence examination is performed with a fibre optic point monitoring system with excitation and detection through the same fibre.

11. The method as recited in claim 1, **characterized** in that the fluorescence examination is performed with a multi-color imaging system with subsequent data handling system.

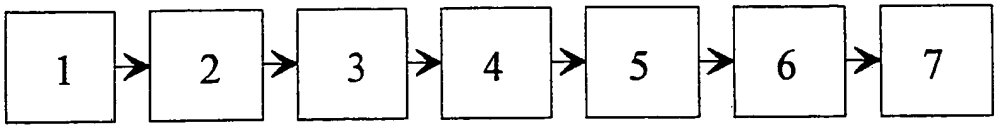

Figure 1

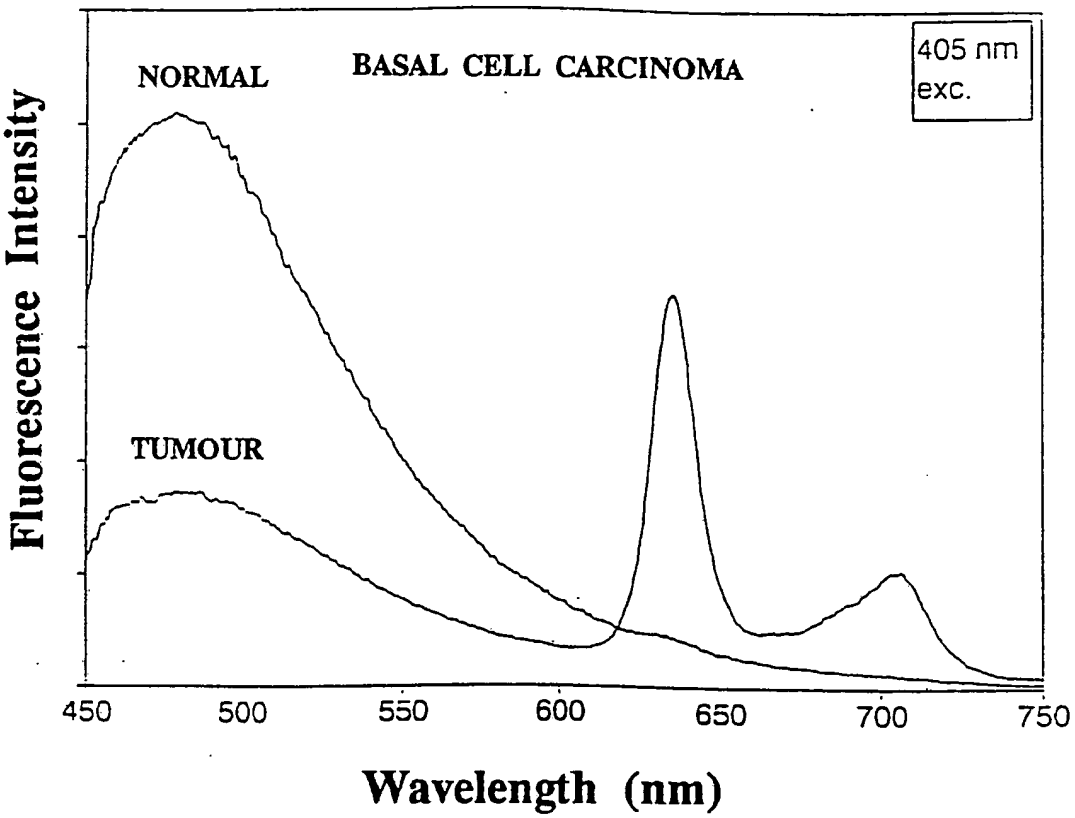
NORMAL
BASAL CELL CARCINOMA
405 nm exc.
TUMOUR
Fluorescence Intensity
450
500
550
600
650
700
750
Wavelength (nm)

Figure 2

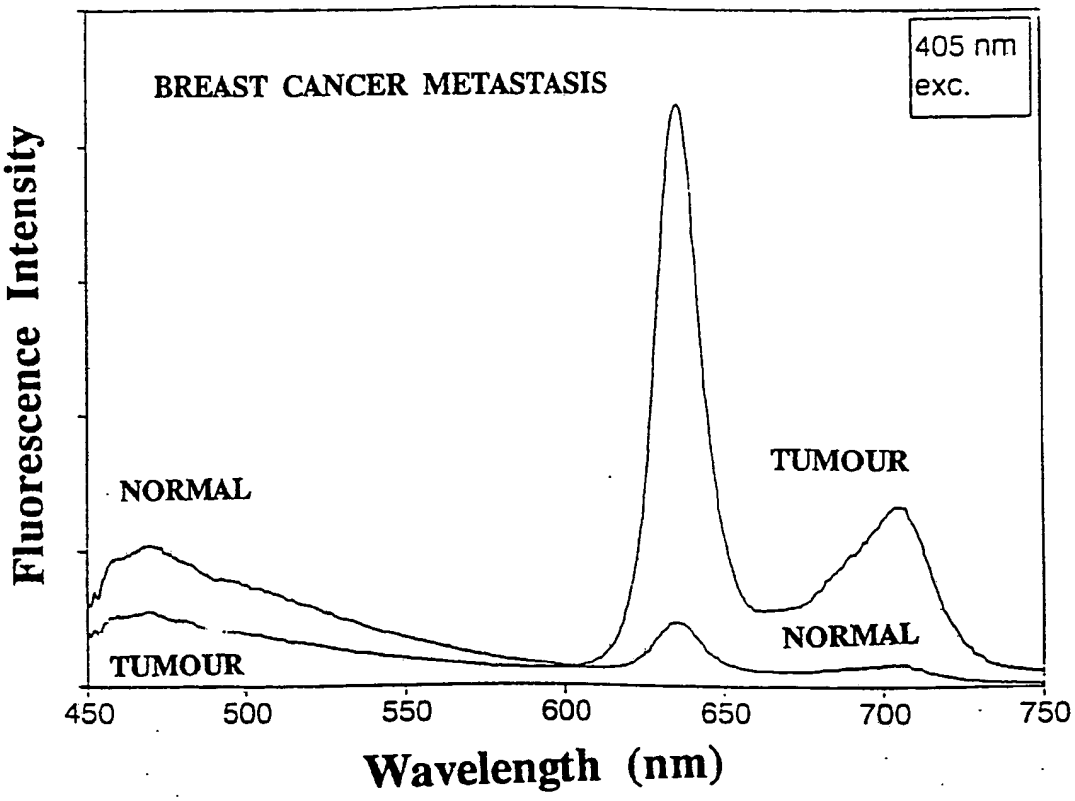
BREAST CANCER METASTASIS
405 nm exc.
Fluorescence Intensity
NORMAL
TUMOUR
TUMOUR
NORMAL
450
500
550
600
650
700
750
Wavelength (nm)

Figure 3

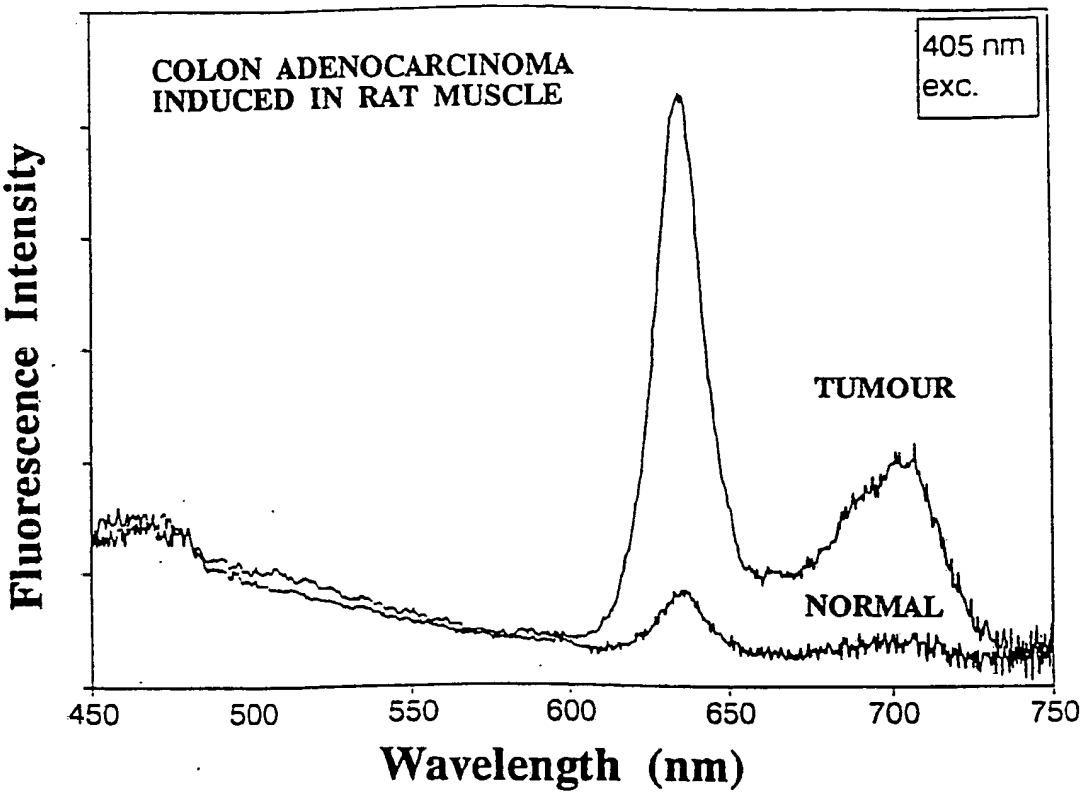
COLON ADENOCARCINOMA
INDUCED IN RAT MUSCLE
405 nm
exc.
TUMOUR
NORMAL
Fluorescence Intensity
450
500
550
600
650
700
750
Wavelength (nm)

Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 92/00879

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 21/64, A61K 31/195, A61K 49/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: A61K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A2, 9101727 (QUEEN'S UNIVERSITY AT KINGSTON), 21 February 1991 (21.02.91), page 7, line 10 - line 18; page 8, line 1 - line 7; page 13, line 23 - page 14, line 14, claims 6-8, 10,11 --	1-11
Y	SE, B, 455646 (RADIANS INNOVA AB), 25 July 1988 (25.07.88), page 5, line 6 - page 6, line 25, abstract --	1-11
A	APPLIED OPTICS, Volume 28, No 12, June 1989, G. C. Tang et al, "Pulsed and cw laser fluorescense spectra from cancerous, normal, and chemically treated normal human breast and lung tissues", see the whole document --	1-11

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 92/00879

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB, A, 2203831 (THE ACADEMY OF APPLIED SCIENCE INC), 26 October 1988 (26.10.88), page 4, line 13 - line 23; page 6, line 1 - line 9, abstract --	1-11
A	WO, A1, 9010219 (STEFAN ANDERSSON-ENGELS ET AL), 7 Sept 1990 (07.09.90), claim 1, abstract --	1-11

International application No.
PCT/SE 92/00879

The claims of the international application appear to relate to subject matter which is not allowable according to Article 17 (2) (a), namely a diagnostic method practised on the human or animal body.

However, an international search report has been established, since a meaningful search could be carried out on the content of the description of the international application.

INTERNATIONAL SEARCH REPORT

Information on patent family members

26/02/93

International application No.
PCT/SE 92/00879

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A2- 9101727	21/02/91	AU-B-	624985	25/06/92
		AU-A-	6034390	11/03/91
		JP-T-	4500770	13/02/92
		NL-A,T-	9021172	01/07/91
		US-A-	5079262	07/01/92
SE-B- 455646	25/07/88	AU-B-	585288	15/06/89
		AU-A-	5065485	15/05/86
		CA-A-	1265232	30/01/90
		DE-A-	3584367	14/11/91
		EP-A,B-	0199767	05/11/86
GB-A- 2203831	26/10/88	NONE		
WO-A1- 9010219	07/09/90	AU-A-	5193790	26/09/90
		CA-A-	2027561	23/08/90
		EP-A-	0411104	06/02/91